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Diatoms in wetlands from the south-west of Western Australia: community structure in relation to pH

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Abstract

A total of 20 wetlands representing three distinct pH groups from the south-west of Western Australia were sampled over three seasons to investigate the relationship between pH and diatom community structure. Multi-dimensional scaling and analyses of similarities were used to identify differences in the diatom community structure according to geographical locations and pH groupings. Regional differences in diatom assemblages were largest between the Collie and Wagerup sites and were associated with varying pH. The largest differences among pH groups were evident between the acidic Group 1 and alkaline Group 3 sites; differences in comparisons were less defined with the circumneutral Group 2 wetlands. BIO-ENV analyses showed that pH was the variable most strongly correlated with diatom distribution patterns during each season. Potential indicator species were identified for each pH group, including *Brachysira brebissonii, Frustulia magaliesmontana, Nitzschia paleaeformis, Brachysira vitrea* and *Staurosira construens* var. *venter.* The results also indicated that diatoms were useful biological indicators of pH in various seasons and can therefore be incorporated into monitoring programs for pH changes in the wetlands of the south-west of Western Australia.

Keywords: acidic, alkaline, circumneutral, south-west Western Australia, wetlands, diatoms

Introduction

The acidification of surface waters has been shown to negatively impact aquatic organisms, with decreases in biodiversity and shifts in community structure commonly reported from different regions of the world (Mason 1991). A further concern for the south-west of Western Australia is the potential contamination of groundwater resources as a result of acidification (McHugh 2004). The use of an effective biological monitoring tool could form an integral part of the management strategy for threatened and acidified waters in the region. Biological monitors such as micro-algae including diatoms could provide an early detection mechanism for the impacts associated with pH decline and allow for the implementation of mitigation procedures.

The sensitivity of diatoms to pH has been clearly demonstrated by several authors such as Hustedt (1938–1939); Cholnoky (1968); Charles (1985); Stokes & Yung (1986); Round (1990) and Watanabe & Asai (2001). However, while diatoms are widely used as pH indicators (van Dam *et al.* 1981) only limited work has been carried out in Western Australia. Studies conducted by John (1993) and Thomas & John (2006) in the south-west of the state investigated diatom community structure in relation to pH but were restricted in scope. Diatom assemblages from 10 sand-mining lakes in Capel were examined by John (1993) while Thomas & John (2006) analysed the diatom community structure of five coal mine void lakes in Collie (pH < 6). Helleren (1993) investigated diatoms as indicators of water quality in 41 wetlands of the Swan Coastal Plain and suggested several species that could potentially be used to indicate acidic waters. However, the majority of sites included in the study were alkaline and only one site was considered to be permanently acidic. In contrast, this study included 20 sites spanning three regions of the south-west. Additionally, wetlands with a greater range of pH were incorporated, including waters that have been acidified due to mining, disturbance of acid sulphate soils and organic acids.

Given that an expanding number of wetlands in Western Australia are under threat of acidification (Department of Environment 2004; McKay & Horwitz 2006) increasing knowledge on the relationship between pH and diatom communities is of growing importance. Therefore, the main objective of this study was to investigate the relationship between environmental variables and diatom communities in wetlands from the south-west of Western Australia with particular reference to pH. A further aim was to identify potential indicator assemblages or species for the different pH classifications. The study also attempted to examine seasonal variability within the distribution patterns of diatoms and assess the impact of seasonality on the effectiveness of diatoms as biological monitors of pH.

Methods

Site locations

A total of 20 sites from three localities within the south-west of Western Australia were sampled in three seasons; during the summer (December–February), winter (June–August) and spring (September–November) of 2001. Nine of the sites, mostly shallow wetlands, were situated in the Perth Metropolitan Region. Five sites were located in the vicinity of Wagerup, a predominantly agricultural area approximately 120 km south of Perth. The remaining six sites were mine void lakes created through coal-mining in the Collie Basin, a sedimentary depression approximately 200 km south-east of Perth (Table 1; Figure 1). The selection of sites from across the three localities provided a comprehensive range of pH and readily accessible sites, a pre-requisite for the seasonal sampling.

pH groupings

The selected sites were classified into three groups according to pH ranges adapted from Foged (1978): Group 1 – acidic (pH < 6.5), Group 2 – circumneutral (pH 6.5–7.5) and Group 3 – alkaline (pH > 7). As a result of the seasonal sampling regime, the pH groupings were flexible, with the number of sites assigned to each group varying in accordance with seasonal fluctuations in pH. The pH groupings of the sites in relation to season have been presented in Table 1 and pH data for each site has been provided in Appendix 1.

Group 1 sites (pH < 6.5) were identified across the three regions and ranged from mine void lakes through to shallow seasonal wetlands. The low pH of these sites was related to factors including mining processes (Commander *et al.* 1994) and the oxidation of acid sulphate soils exposed by decreasing groundwater levels (McHugh 2004). Organic acids are also likely to have contributed, having been associated with low pH waters during previous studies in the south-west (Schmidt & Rosich 1993; Kinnear & Garnett 1999).

Group 2 sites (pH 6.5–7.5) were also identified across the three regions and ranged from shallow wetlands to mine void lakes. Land-uses in the vicinity of the Group 2 sites were generally related to urban development or agriculture.

Group 3 incorporated alkaline wetlands from two of the regions (Wagerup and the Perth Metropolitan Region). The sites were generally shallow in nature with surrounding land-uses including residential, industrial, pastoral and water collection. Substrate type and nutrient enrichment were two of the factors likely to have contributed to the alkaline pH of various Group 3 wetlands.

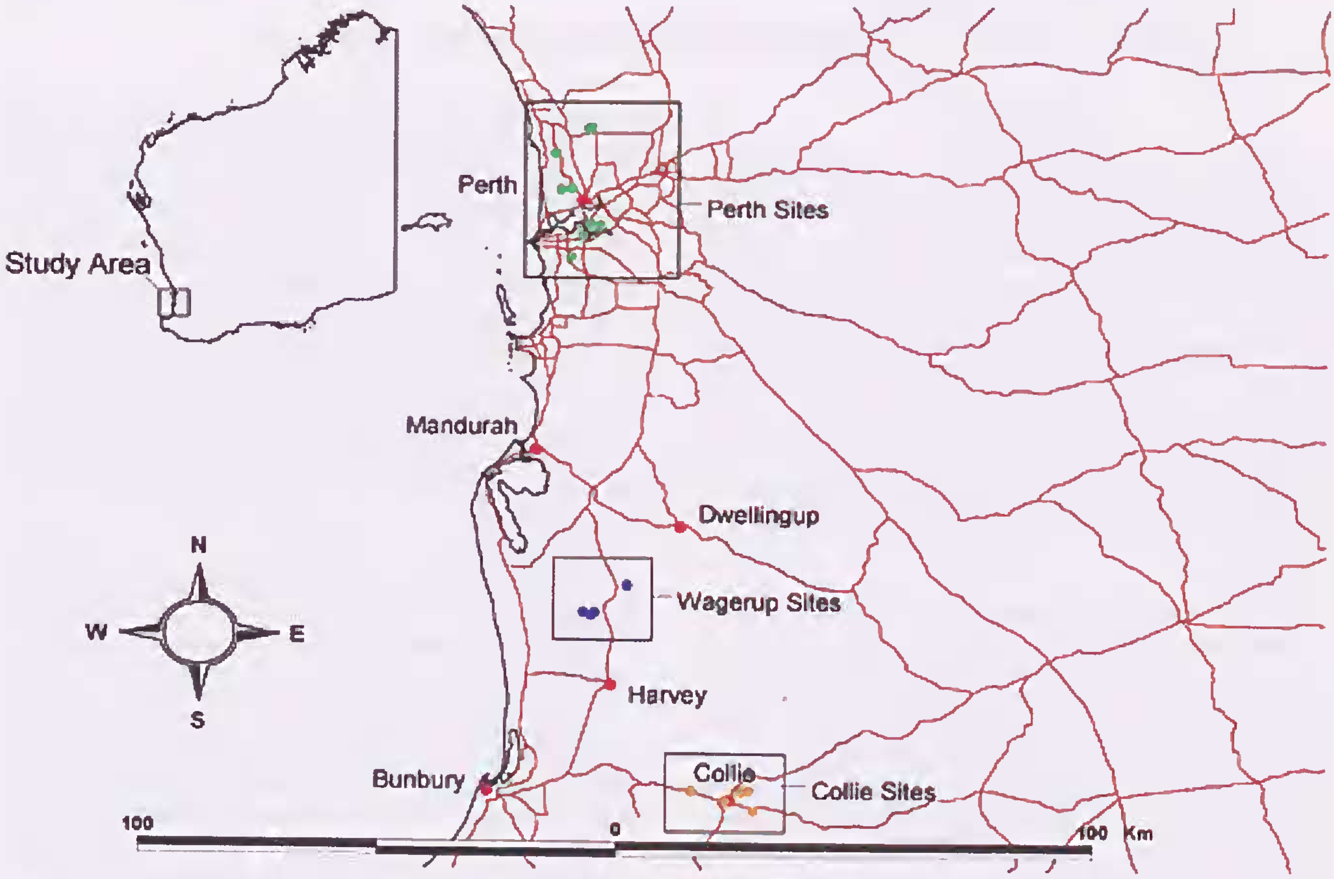

Figure 1. Location of the 20 sites in the south-west of Western Australia selected for the study, situated in the Perth Metropolitan Region, Wagerup and the Collie Basin. The closest neighbouring towns to each regional location are also shown.

Table 1

Site names, codes, locations, GPS coordinates and seasons sampled for the 20 study sites from the south-west of Western Australia. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample.

Site Name	Site Code	Site Location	GPS Coordinates	Season	pH Group
Bibra Lake	Su1	Perth Metropolitan	32°05.19s 115°49.38e	Summer	3
	Wi1			Winter	3
	Sr1			Spring	3
Black Diamond	Su2	Collie Basin	33°20.33s 116°05.58e	Summer	1
	Wi2			Winter	1
	Sr2			Spring	1
Blind Roo A	Su3	Wagerup	32° 55.20s 115° 51.23e	Summer	1
	Wi3			Winter	2
	Sr3			Spring	1
Blind Roo B	Su4	Wagerup	32° 55.21s 115° 51.25e	Summer	3
	Wi4			Winter	3
	Sr4			Spring	3
Blue Gum Lake	Su5	Perth Metropolitan	32°02.20s 115°50.90e	Summer	3
	Wi5			Winter	3
	Sr5			Spring	3
Blue Waters	Su6	Collie Basin	33°20.24s 116°13.16e	Summer	1
	Wi6			Winter	1
	Sr6			Spring	1
Ewington 2	Su7	Collie Basin	33°20.48s 116°12.02e	Summer	1
	Wi7			Winter	1
	Sr7			Spring	1
Exelby Wetland	Su8	Wagerup	32° 55.64s 115° 52.23e	Summer	3
	Wi8			Winter	2
	Sr8			Spring	2
Gnangara Lake	Su9	Perth Metropolitan	31°46.97s 115°51.96e	Summer	1
	Wi9			Winter	1
	Sr9			Spring	1
Herdsman Lake	Su10	Perth Metropolitan	31°55.71s 115°48.03e	Summer	3
	Wi10			Winter	3
	Sr10			Spring	3
Knapping Wetland	Su11	Wagerup	32° 55.27s 115° 52.69e	Summer	3
	Wi11			Winter	2
	Sr11			Spring	3
Kurrajong Village Lake	Su12	Perth Metropolitan	32°00.76s 115°53.19e	Summer	2
	Wi12			Winter	1
	Sr12			Spring	2
Lake Monger	Su13	Perth Metropolitan	31°55.50s 115°49.45e	Summer	3
	Wi13			Winter	3
	Sr13			Spring	3
Lake Moyanup	Su14	Wagerup	32°51.41s 115°56.99e	Summer	3
	Wi14			Winter	2
	Sr14			Spring	3
Lakelands	Su15	Perth Metropolitan	31°50.55s 115°47.29e	Summer	1
	Wi15			Winter	2
	Sr15			Spring	1
Neil McDougall Park	Su16	Perth Metropolitan	32°00.45s 115°51.83e	Summer	3
	Wi16			Winter	3
	Sr16			Spring	3
Stockton Lake	Su17	Collie Basin	33°23.13s 116°13.75e	Summer	1
	Wi17			Winter	1
	Sr17			Spring	1
Stockton Tailings Pond	Su18	Collie Basin	33°23.13s 116°13.74e	Summer	1
	Wi18			Winter	1
	Sr18			Spring	1
Tuscan Park	Su19	Perth Metropolitan	31°47.12s 115°51.75e	Summer	1
	Wi19			Winter	2
	Sr19			Spring	2
Wallsend Lake	Su20	Collie Basin	33°21.65s 116°09.99e	Summer	1
	Wi20			Winter	2
	Sr20			Spring	2

Environmental variables

The environmental variables of pH, electrical conductivity (μS cm^{-1}), salinity (ppm), temperature (°C) and dissolved oxygen (mg L^{-1}) were recorded from each site during the three seasons. The peripheral vegetation of each site was observed and assigned a score between 1–5, with 1 given to sparsely vegetated sites and 5 representing the most densely vegetated sites. The environmental data has been presented in Appendix 1.

Periphytic diatom sampling

Diatom samples were collected using an artificial substrate collector known as the JJ Periphytometer (John 1998). The periphytometers, fitted with 10 glass microscope slides and exposed to colonizers vertically, were employed to ensure uniform collection of diatoms. This method also avoids the problem of collecting dead cells and allows the diatom assemblages to be related to the ambient environmental conditions. Wire or 20 lb fishing line was used to secure the periphytometers to submerged structures such as a stake or tree root, ensuring the devices were well immersed. The periphytometers were retrieved after approximately 14 days of immersion, allowing sufficient time for the development of a climax community (John 1998). The slides removed from each periphytometer were placed in vials containing deionised water and preserved with the addition of 5–10 ml of Transeau's Algal Preservative (6:3:1 deionised water, ethyl alcohol and formalin).

Permanent slide preparation of diatom samples

Preparation of the diatom samples followed the techniques outlined in John (1998). The film of periphyton on both sides of the 10 slides retrieved from the JJ periphytometer was scraped into a mixture of deionised water and Transeau's Algal Preservative. Between 10–20 ml of the sample was placed in a 100 ml beaker and digested with equal amounts of Nitric Acid (70 %) and deionised water, on a hot plate set at 80 °C. After cooling, the suspension was diluted with deionised water and centrifuged for five minutes at 3500 rpm. The supernatant was decanted off to remove the acid, leaving a pellet of diatom frustules, and deionised water was added to resuspend the frustules. The centrifugation process was repeated a further four times to remove all traces of acid.

Aliquots of 100 to 1000 μl of the resuspended samples were pippetted onto glass coverslips placed on a hot plate (60 °C). The concentration varied according to the density of diatoms in the sample, with deionised water added when aliquots of less than 1000 μl were used. The even distribution of diatom frustules on the coverslips was achieved through gentle stirring of the sample. Upon evaporation, the coverslips with the dried diatom samples were inverted and gently pressed down onto clean glass slides with 4–5 drops of the mounting medium Naphrax (refractive index 1.74). The slides were placed on the hotplate to boil until the solvent present in the Naphrax had evaporated. The slides were subsequently removed, allowing the medium to cool and solidify. Three permanent slides were prepared for each sample.

Diatom enumeration

The slides were examined under oil immersion at 1000x magnification. Depending on the density of diatoms, between 100–350 diatom valves were counted and identified from each sample. Identification was to species level where possible and the relative frequencies of each taxon were determined for statistical analyses. Diatoms were identified using specialised literature (Patrick & Reimer 1966; Foged 1974; Patrick & Reimer 1975; Foged 1978; John 1983; Hustedt & Jensen 1985; Gasse 1986; Krammer & Lange-Bertalot 1986; Holland & Clarke 1989; Lange-Bertalot & Moser 1994; Ehrlich 1995; Snoeijs & Balashova 1998; John 1998; Camburn & Charles 2000; John 2000a; Siver *et al.* 2005). Diatom nomenclature generally conformed to Fourtanier & Kociolek (1999). Photomicrographs of diatoms were taken under oil immersion at 1000x magnification. The diatom slides have been deposited in the International Diatom Herbarium at the Department of Environmental and Aquatic Sciences, Curtin University of Technology.

Data analysis

Diatom community composition was investigated using multivariate analyses from the software package PRIMER 5.0 for Windows Version 5.2.9 (PRIMER-E Ltd 2002). Non-metric multi-dimensional scaling (MDS) ordinations (Kruskal & Wish 1978) were employed to identify groups of sites with similar diatom community structure. Species abundance data were square-root transformed and the Bray Curtis similarity measure (Bray & Curtis 1957) was used to construct similarity matrices. The significance of trends in diatom distribution was determined using one way analysis of similarity or ANOSIM (Clarke & Green 1988). A maximum of 999 permutations were used to calculate the probability of the observed values for each analysis. The BIO-ENV procedure (Clarke & Ainsworth 1993) identified the combinations of environmental variables which provided the best matches of biotic and environmental matrices. This was achieved using the Spearman rank correlation coefficient (ρ_s). Significance was accepted at $p < 0.05$ and $p < 0.01$.

Results

Diatom taxa

A total of 154 diatom taxa from 44 genera were recorded across the three seasons (Table 2). Over 110 taxa were identified from the summer and winter samples (115 and 113 respectively) with 106 present in the spring collection. The genus *Nitzschia* displayed the highest species richness in each season. *Achnanthidium*, *Navicula* and *Gomphonema* were also prominent although abundance varied. During the spring collection *Pinnularia* was also among the common genera.

Dominant taxa of the pH groups

Diatom taxa that were observed in at least two sites per wetland pH group with an abundance of at least 10 % were classified as dominant (Table 3). The species *Brachysira brebissonii* (Figure 2a) *Frustulia magaliesmontana* (Figure 2b), *Navicula* aff. *cari* and *Nitzschia paleaeformis*

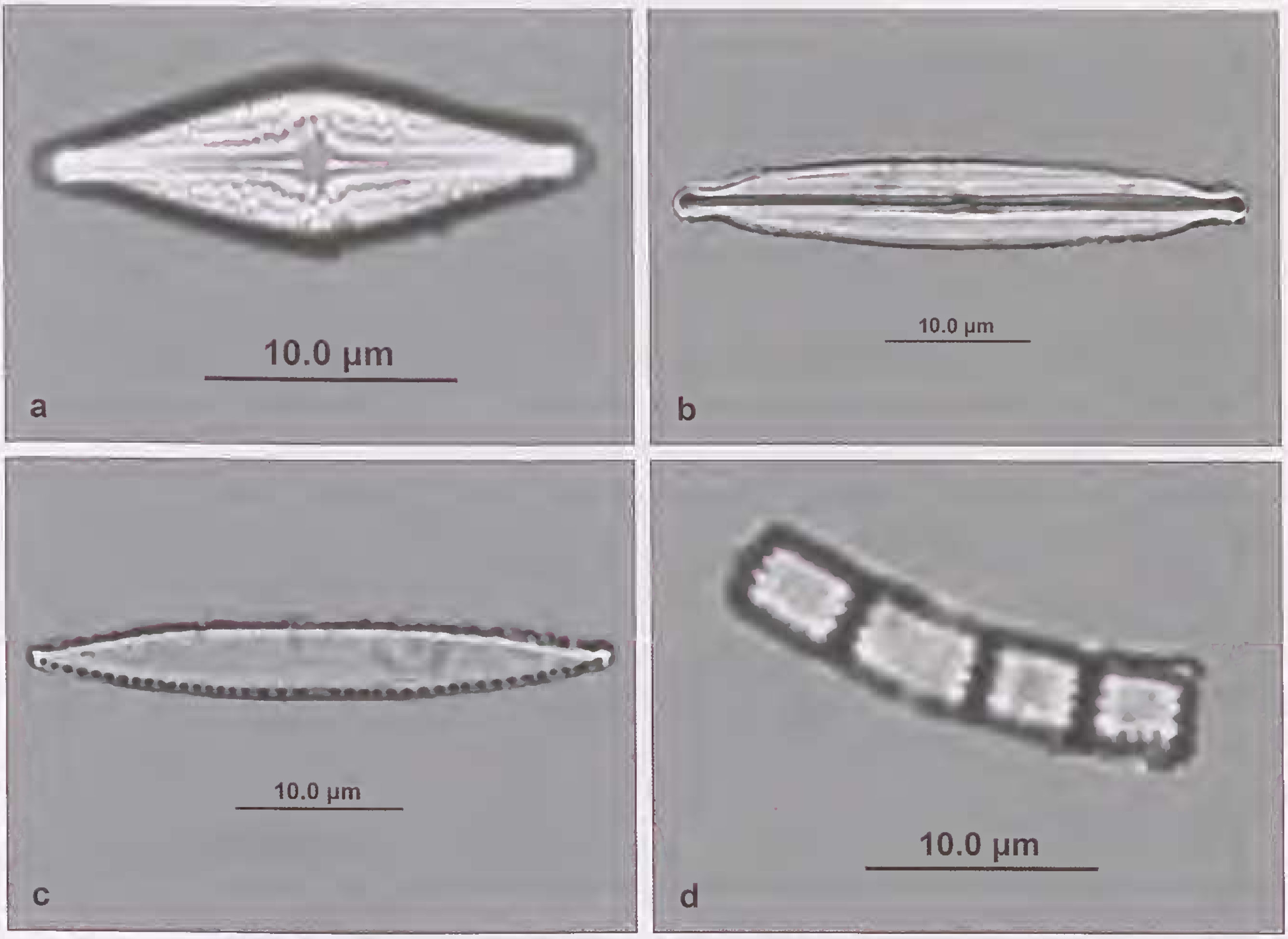

Figure 2. Examples of potential diatom indicator species a) *Brachysira brebissonii,* b) *Frustulia magaliesmontana,* c) *Nitzschia paleaeformis,* d) *Staurosira construens* var. *venter* (chain of frustules in girdle view).

Table 2

List of genera identified from the 20 study sites in the south-west of Western Australia over the three seasons and the number of species recorded from each genus.

Genus	Species Number	Genus	Species Number
Achnanthes	1	*Gomphonema*	13
Achnanthidium	11	*Gyrosigma*	1
Amphora	6	*Hantzschia*	2
Aulacoseira	1	*Hippodonta*	1
Bacillaria	1	*Luticola*	1
Brachysira	5	*Mastogloia*	2
Caloneis	1	*Navicula*	13
Cocconeis	3	*Neidium*	3
Craticula	2	*Nitzschia*	18
Ctenophora	1	*Pinnularia*	9
Cyclotella	3	*Placoneis*	1
Cylindrotheca	1	*Planothidium*	1
Cymbella	2	*Pseudostaurosira*	1
Diadesmis	1	*Rhopalodia*	2
Encyonema	5	*Sellaphora*	2
Encyonopsis	1	*Stauroneis*	6
Epithemia	2	*Staurosira*	2
Eunotia	8	*Surirella*	3
Fallacia	1	*Synedra*	3
Fragilaria	6	*Tabellaria*	1
Fragilariforma	1	*Tabularia*	1
Frustulia	5	*Tryblionella*	1

(Figure 2c) were commonly recorded from the acidic Group 1 wetlands throughout the study. *Brachysira styriaca, Eunotia bilunaris* and *Eunotia pectinalis* var. *minor* also displayed a dominant presence in this group, although the seasons varied.

The dominant taxa of Group 2 displayed some overlap with alkaline Group 3. While *Achnanthidium minutissimum* and *Gomphonema parvulum* were classified as abundant in the Group 2 winter samples, both species displayed a dominant presence in the Group 3 wetlands in two or more seasons. *Brachysira vitrea* was the only taxon that occurred abundantly in the winter circumneutral wetlands alone. As none of the taxa recorded from the Group 2 wetlands during spring exceeded 10 % abundance in at least two sites, species dominance was not established.

Nine diatom taxa were classified as abundant in the alkaline Group 3 wetlands, the majority of which were rarely recorded from the other wetland groups. *Gomphonema parvulum* and *Staurosira construens* var. *venter* (Figure 2d) were commonly identified from the wetlands of Group 3 throughout the study, while species such as *Cocconeis placentula, Pseudostaurosira brevistriata, Encyonopsis microcephala* and *Nitzschia palea* were seasonally abundant (Table 3).

Table 3

Summary of the dominant diatom taxa from each pH group of wetlands sampled in the south-west of Western Australia over the three seasons. Taxa included were present in at least two sites within a pH group with an abundance of > 10 %. Group 1 represents acidic sites, Group 2 denotes circumneutral sites and Group 3 represents alkaline sites.

Taxa	Summer[1]		Winter			Spring		
	Group 1	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Achnanthidium minutissimum		X		X				X
Brachysira brebissonii	X		X			X		
Brachysira styriaca						X		
Brachysira vitrea				X				
Cocconeis placentula								X
Encyonopsis microcephala		X						
Eunotia bilunaris			X					
Eunotia pectinalis var. minor	X		X					
Frustulia magaliesmontana	X		X			X		
Gomphonema parvulum		X		X	X			X
Navicula aff. *cari*	X		X			X		
Navicula cryptocephala					X			X
Nitzschia palea								X
Nitzschia palea var. 1								X
Nitzschia paleaeformis	X		X			X		
Pseudostaurosira brevistriata		X						
Staurosira construens var. venter		X			X			X

[1] Group 2 was excluded from the summer results due to the limited sample size.

Multivariate analyses

The ordinations of the sites based on the similarities in species assemblages displayed moderate levels of separation for each of the seasons (Figure 3a–c). Ordination of the sites according to regional location (Figure 4a–c) demonstrated that Collie sites generally clustered together, although the groupings were not discrete. In contrast, the Perth wetlands displayed two separate groupings. Five of the sites tended to cluster near the Wagerup wetlands, while the remaining four contained diatom assemblages similar to the Collie sites. The Wagerup sites grouped to the right of the axis during each season, displaying a reasonably strong separation from the Collie sites.

An overlay of symbols representing the three wetland pH groupings (Figure 4d–f) demonstrated that the acidic Group 1 wetlands were generally well separated from the alkaline Group 3 wetlands in each season. The Group 1 wetland of Blind Roo A (Site 3) was the only exception, clustering with the Group 3 wetlands during summer and appearing relatively close in ordination space during spring. The circumneutral Group 2 wetlands mostly occupied an intermediate position and displayed less defined clustering than either the Group 1 or Group 3 wetlands (Figure 4).

One way analyses of similarities (ANOSIM) conducted on the seasonal diatom data detected significant differences in the community structure of the regional location groups during each season ($p < 0.05$) (Table 4). The diatom assemblages of the Collie and Wagerup

Table 4

Results from one-way analyses of similarities (ANOSIM) and pairwise tests on Bray-Curtis similarities of square root transformed diatom abundance data from the regional location groups of wetlands sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	Region	R	Probability
Summer	All Regions	0.32	**< 0.01**
	Perth Metropolitan Region, Collie	0.19	> 0.05
	Perth Metropolitan Region, Wagerup	0.21	> 0.05
	Collie, Wagerup	0.78	**< 0.01**
Winter	All Regions	0.21	**< 0.05**
	Perth Metropolitan Region, Collie	0.15	> 0.05
	Perth Metropolitan Region, Wagerup	0.01	> 0.05
	Collie, Wagerup	0.72	**< 0.01**
Spring	All Regions	0.31	**< 0.01**
	Perth Metropolitan Region, Collie	0.26	**< 0.05**
	Swan Coastal Plain, Wagerup	0.12	> 0.05
	Collie, Wagerup	0.70	**< 0.01**

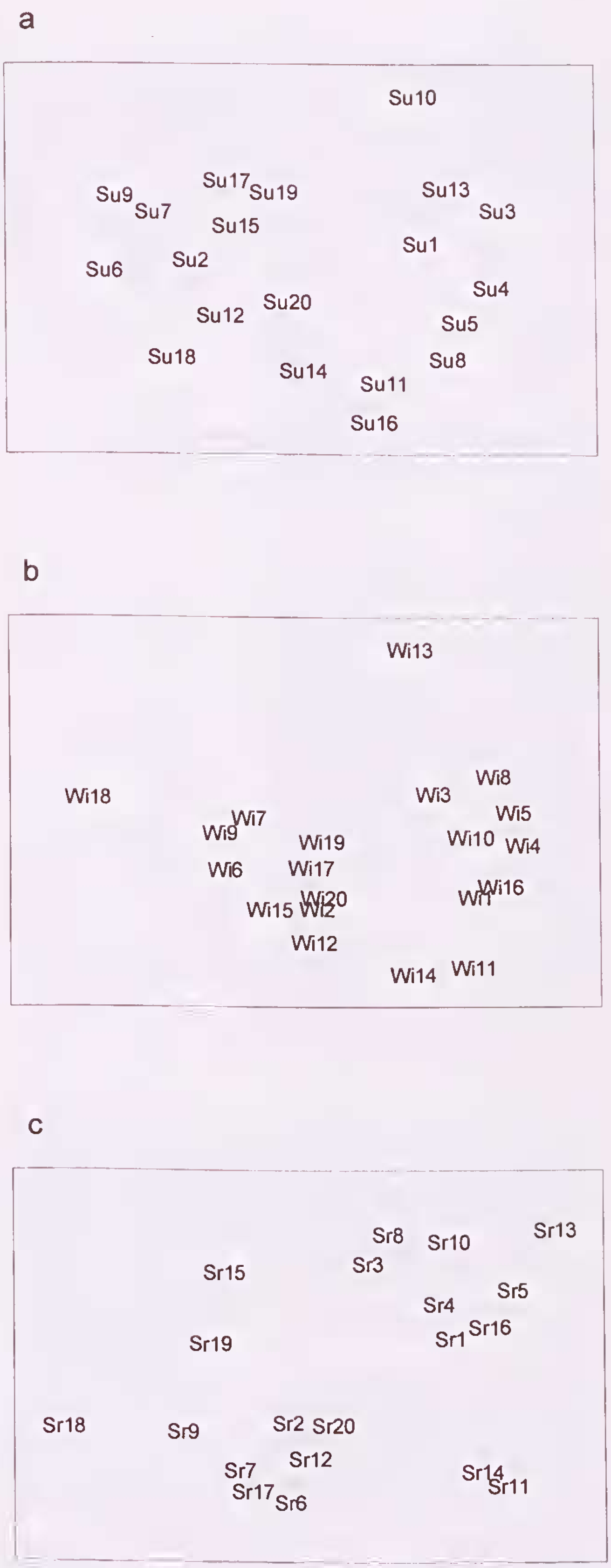

Figure 3. Two dimensional multi-dimensional scaling (MDS) ordination of square root transformed diatom abundance data with site codes superimposed. a) MDS plot of summer data, stress = 0.14. b) MDS plot of winter data, stress = 0.11. c) MDS plot of spring data, stress = 0.14. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample.

Table 5

Results from one-way analyses of similarities (ANOSIM) and pairwise tests on Bray-Curtis similarities of square-root transformed diatom abundance data from the pH groups of wetlands sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	pH Group[1]	R	Probability
Summer	Group 1, Group 3	0.64	**< 0.01**
Winter	All Groups	0.46	**< 0.01**
	Group 1, Group 2	0.25	**< 0.05**
	Group 1, Group 3	0.82	**< 0.01**
	Group 2, Group 3	0.24	**< 0.05**
Spring	All Groups	0.51	**< 0.01**
	Group 1, Group 2	0.10	> 0.05
	Group 1, Group 3	0.76	**< 0.01**
	Group 2, Group 3	0.46	**< 0.05**

[1] Group 2 not included in summer comparison due to the limited sample size.

regions were significantly different to each other in each season with relatively high R values (≥ 0.70) suggesting that the differences between these regional locations were strong. The Collie and Perth Metropolitan sites were significantly different during spring only and the relatively low R value (0.26) during this season suggested that the separation between these groups was not strong.

ANOSIM tests on the summer and winter data also established significant differences in the diatom community structure of each wetland pH group ($p < 0.05$) (Table 5). Comparisons of the acidic Group 1 wetlands and the alkaline Group 3 wetlands revealed the greatest differences during each season ($R > 0.60$). Other groups were significantly different during winter although the low R values ($R < 0.50$) suggest that the separation of these groups was not as strong. The spring ANOSIM generally displayed similar findings to the other seasons. The comparison between Group 1 and Group 2 was the only exception, with no significant differences detected between the diatom communities of the two groups during this season (Table 5).

The BIO-ENV analyses between environmental variables and diatom abundance data determined that the strongest correlation in each of the seasons was produced by a single parameter (Table 6). The variable of pH displayed the strongest correlation with community structure during summer and spring and achieved the same correlation as the two variable combination of pH and dissolved oxygen during winter ($\rho_s > 0.60$), supporting the relationship illustrated in Figure 4d–f.

Discussion

Diatom flora

A total of 154 diatom taxa were recorded during the study, spanning three seasons. The genera represented by the most species were consistent with the findings of previous Western Australian studies and those from other areas of Australia. For example, *Achnanthidium*, *Gomphonema*, *Navicula* and *Nitzschia* were identified as

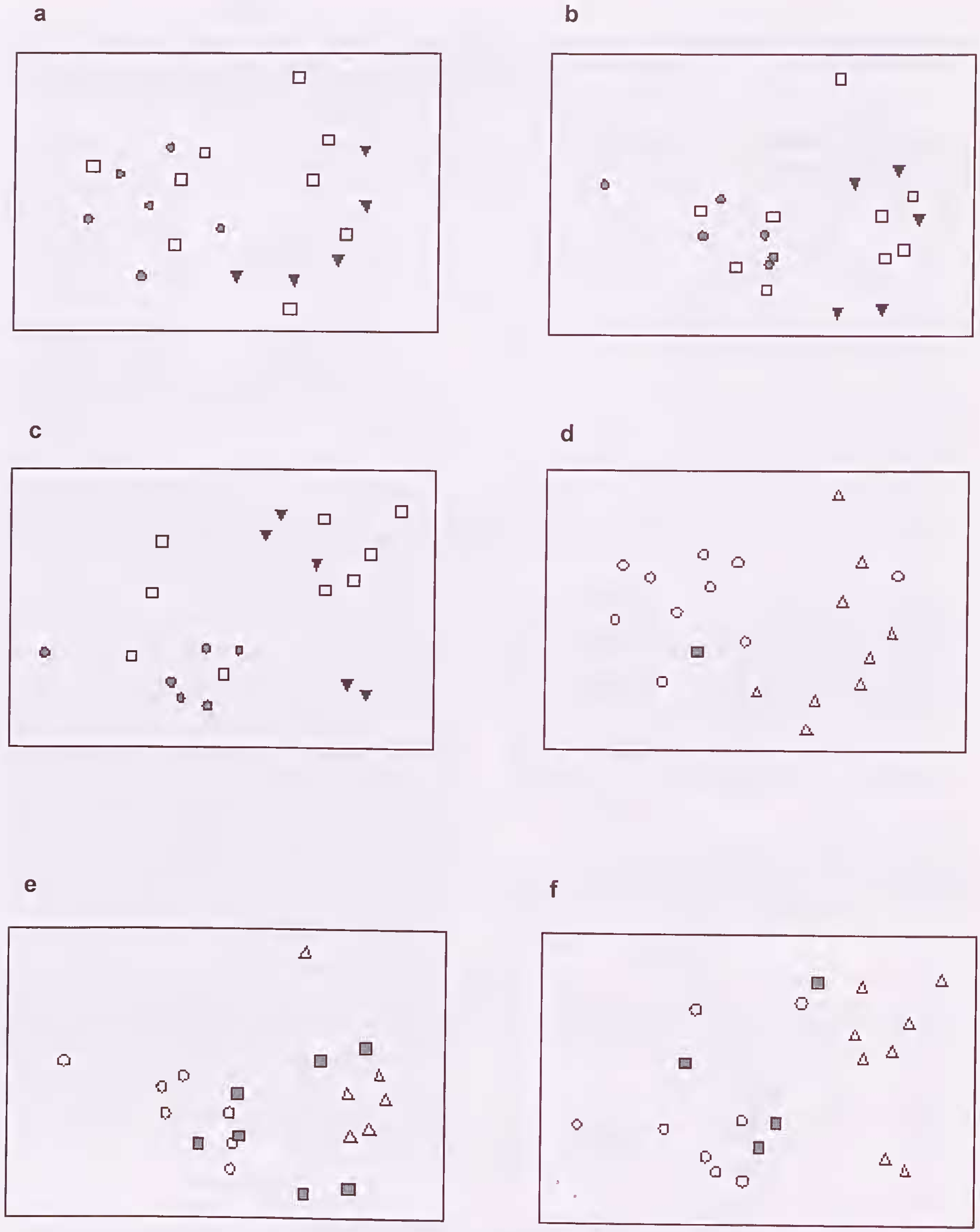

Figure 4. Two dimensional multi-dimensional scaling (MDS) of square-root transformed diatom abundance data. a) MDS plot of summer data with overlay of regional locations, stress = 0.14. b) MDS plot of winter data with overlay of regional locations, stress = 0.11. c) MDS plot of spring sample with overlay of regional locations, stress = 0.14. □ represent Perth Metropolitan sites, ● represent Collie Basin sites and ▼ represent Wagerup sites. d)–f) MDS plots of the summer, winter and spring data with pH groups superimposed. O represent the acidic Group 1 sites, ■ represent the circumneutral Group 2 sites and Δ represent the alkaline Group 3 sites.

Table 6

BIO-ENV results giving the combinations of environmental variables with the highest rank correlations between the environmental variables and the square root transformed diatom similarity matrices as measured by Spearman rank correlation (ρ_s). A correlation cut-off of $\rho_s < 0.40$ was applied. The strongest correlation is presented in bold type. Veg = vegetation score, EC = electrical conductivity (µS cm^{-1}), Temp = temperature (°C), DO = dissolved oxygen (mg L^{-1}).

Season	Number of Variables	Variables	ρ_s
Summer	1	pH	**0.62**
	2	pH, DO	0.58
	3	pH, Temp, DO	0.52
	2	pH, Temp	0.52
	3	pH, EC, DO	0.51
	4	pH, EC, Temp, DO	0.49
	2	pH, EC	0.48
	3	Veg, pH, DO	0.44
	4	Veg, pH, Temp, DO	0.43
	3	pH, EC, Temp	0.43
Winter	1	pH	**0.62**
	2	pH, DO	0.62
	3	pH, EC, DO	0.55
	3	pH, Temp, DO	0.55
	2	pH, Temp	0.52
	4	pH, EC, Temp, DO	0.52
	2	pH, EC	0.51
	3	Veg, pH, DO	0.49
	3	pH, EC, Temp	0.48
	4	Veg, pH, EC, DO	0.47
Spring	1	pH	**0.63**
	2	pH, DO	0.48
	2	pH, Temp	0.45
	2	pH, EC	0.44
	2	pH, Veg	0.41
	3	pH, Temp, DO	0.40

some of the most species-rich taxa in a study on the classification of urban streams in the Perth Metropolitan Region (John 2000b). Sonneman *et al.* (2001) also identified *Nitzschia, Navicula, Gomphonema* and *Achnanthes* as the genera with the highest number of species during a study in Melbourne, Victoria. The aforementioned genera were also commonly recorded from streams in New South Wales and Victoria (Chessman *et al.* 1999).

Separation of the sites into the three wetland pH groups identified 17 dominant taxa. The alkaline wetlands of Group 3 contained the highest number of abundant species while the circumneutral Group 2 wetlands had the lowest number. This may be indicative of the small sample size of Group 2 wetlands throughout the study rather than a lack of species favouring circumneutral conditions. Alternatively, the circumneutral waters may have provided favourable conditions for a greater diversity and more even distribution of diatom taxa (Patrick & Strawbridge 1963). It is also important to note that the circumneutral or Group 2 classification had a very limited pH range (pH 6.5–7.5) in comparison to the acidic and alkaline wetland groups (pH < 6.5 and > 7.5 respectively), potentially reducing the number of species that displayed a clear preference for circumneutral waters.

The acidic Group 1 sites were consistently dominated by diatoms including *Brachysira brebissonii, Frustulia magaliesmontana* and *Nitzschia paleaeformis* which could potentially be used as indicator species. The occurrence of *Brachysira brebissonii* in acidic waters has been well documented (van Dam *et al.* 1981; DeNicola 1986; Vinebrooke *et al.* 2003). Cholnoky (1968) reported that the optimum pH for the species was 5.2 and Gasse (1986) noted that a low pH appeared to be the most important ecological variable for the species. *Frustulia magaliesmontana* has previously been reported from dystrophic acidic lakes in the western region of Tasmania (Vyverman *et al.* 1996) and from acidic sand-mine lakes in Capel, southwestern Australia (pH < 4) (John 1993). *Nitzschia paleaeformis* was another taxon found commonly in Capel sand-mine lakes (John 1993). Further records of the species include a study on Japanese water bodies (pH 2.6–3.9) (Watanabe & Asai 2004) and a study on lentic heathland waters in Belgium (Denys & van Straaten 1992).

Brachysira styriaca and *Eunotia bilunaris* were among the taxa that commonly contributed to the community structure of Group 1 wetlands in at least one season. *Brachysira styriaca* has been recorded from countries including Iceland and the United States of America and appears to favour waters with pH readings of < 7 (Foged 1974; Siver *et al.* 2005). *Eunotia bilunaris* has been described by Patrick & Reimer (1966) as a frequent inhabitant of acidic waters.

Brachysira vitrea was abundant in the Group 2 wetlands only and this apparent preference for circumneutral waters is supported by the findings of other studies. Round (1990) highlighted the association of *Brachysira vitrea* with pH readings at the higher end of the spectrum (mean pH = 6.6) in a study of Welsh lakes ranging from pH 4.4–6.8. While a study of mostly acidic lakes in Florida reported that *Brachysira vitrea* was only recorded from a single lake, significantly less acidic than the other sites (mean pH = 7) (Shayler & Siver 2004). Additionally, Coring (1996) lists the taxon as being more indicative of dystrophic (humic acid) conditions rather than anthropogenic acidification.

Achnanthidium minutissimum was one of the two dominant taxa that overlapped between the circumneutral wetlands and the alkaline sites of Group 3. The species is cosmopolitan in distribution (Foged 1979) and in terms of pH preference, appears to vary according to the region. During studies in eastern Australia and New Zealand, Foged (1978; 1979) classified the taxon as alkaliphilous while Gasse (1986) commonly recorded the species in African waters ranging from weakly acidic to alkaline (pH = 6.0–8.5). Cholnoky (1968) stated that the optimum pH for *Achnanthidium minutissimum* lay between 7.5–7.8 and Patrick and Reimer (1966) found that the species occurred most frequently between pH 6.5–9.0. Similarly, in the current study *Achnanthidium minutissimum* was most abundant in wetlands with a pH > 6.5.

Gomphonema parvulum was the second taxon to occur frequently in both Group 2 and Group 3 wetlands. Similar to *Achnanthidium minutissimum*, the pH preference of the former appears less specific than many other species. Schoeman (1973) reported that *Gomphonema parvulum* had the ability to tolerate large pH fluctuations, commonly finding it in strongly alkaline and neutral waters and recording low frequencies from some acidic waters in Lesotho, Africa. Foged (1974; 1979) and Gasse (1986) noted that the taxon generally occurs in circumneutral to alkaline waters, supporting the dominance of the species in both the Group 2 and Group 3 wetlands in this study.

Aside from *Gomphonema parvulum, Staurosira construens* var. *venter* was the only taxon to maintain a dominant presence in the Group 3 wetlands during all three seasons and may have potential applications as an indicator species. The species is known to be widely distributed (Patrick & Reimer 1966) and has been identified from regions including Russia (Laing & Smol 2000) and the United States of America (Camburn & Charles 2000). Previous records in Australia include streams and wetlands in Victoria (Blinn & Bailey 2001; Gell *et al.* 2002), wetlands on the Swan Coastal Plain (Helleren 1993) and the Swan and Canning Rivers in Perth (John 1983). Research from East Africa suggested that *Staurosira construens* var. *venter* favours waters of circumneutral to alkaline waters, although small numbers were also recorded from slightly acidic sites (Gasse 1986).

Cocconeis placentula, Pseudostaurosira brevistriata, Encyonopsis microcephala and *Nitzschia palea* were among the taxa abundant in a particular season. *Cocconeis placentula* was described by Cholnoky (1968) as a good indicator of moderately alkaline conditions (pH optimum of about 8) and *Nitzschia palea* was calculated to have an optimum pH of 8.4. *Encyonopsis microcephala* and *Pseudostaurosira brevistriata* have both been recorded from waters of varying pH conditions (Schoeman 1973; Hustedt & Jensen 1985), although they tend to occur most commonly in circumneutral to alkaline waters (Foged 1974; Gasse 1986).

Diatom community structure

Multi-dimensional scaling and analyses of similarities identified some significant differences in the community composition of diatoms in the different regional locations. The Collie wetlands, while not discretely grouped, tended to cluster together in ordination space and were generally well separated from the Wagerup wetlands. This was supported by analyses of similarities which detected significant differences in the diatom assemblages of the two regions during each season. The differences in community structure were likely to be partly related to pH, with the Collie lakes generally exhibiting comparatively lower pH values, attributed to past mining processes (Commander *et al.*1994).

The Perth Metropolitan wetlands tended to separate into two groups. Gnangara Lake (Site 9), a shallow wetland with low pH linked to the oxidation of acid sulphate soils, grouped near the Collie sites as did acidic and circumneutral metropolitan sites including Lakelands (Site 15), Tuscan Park (Site 19) and Kurrajong Village Lake (Site 12). In contrast, alkaline Perth sites such as Lake Monger (Site 13), Bibra Lake (Site 1) and Blue Gum Lake (Site 5) generally clustered near the mostly alkaline Wagerup sites. Geology is likely to be one of the factors which contributed to the alkaline nature of the Perth sites, with Lake Monger located on the limestone rich Spearwood Dune system (Lund and Davis 2000) and Blue Gum and Bibra Lakes (as part of the eastern Beeliar wetlands) bordering the Spearwood Dune system (Bennett Brook Environmental Services 2004). In addition, wetlands such as Bibra Lake and Lake Monger are eutrophic (Cheal *et al.*1993) and may display elevated pH in response to high levels of photosynthesis (Schmidt & Rosich 1993).

MDS and analyses of similarities also detected significant differences in the community structure of diatoms in the three wetland pH groups, suggesting that pH was an important factor contributing to the separation of the sites. The largest differences in community structure were evident between the acidic Group 1 and alkaline Group 3 wetlands. Dissimilarities between the dominant taxa of the two groups of wetlands further supported the differences displayed in the overall community structure.

The primary exception to the otherwise strong separation of the two groups of wetlands was the generally acidic wetland of Blind Roo A (Site 3). This site, along with other Wagerup wetlands including Blind Roo B (Site 4) was originally created through clay extraction. However, in contrast to the mostly alkaline nature of the other Wagerup sites, Blind Roo A was relatively dark in colour and had a comparatively low pH, attributed to humic acid derived from high levels of vegetative material. Despite this, the overall community composition was atypical of acidic wetlands, suggesting that variables other than pH were the over-riding factors in the diatom community structure. Taking the history of the site into consideration, it seems likely that unmeasured factors linked to substrate type may have influenced the diatom assemblages present. Additionally, the Wagerup sites are situated on pastoral land and surrounding land-use practices have potentially exposed the wetlands to impacts such as nutrient enrichment (Harper 1992). For example *Gomphonema parvulum*, a species able to tolerate a range of pH (Schoeman 1973) and known to occur in waters with high nutrient levels (Patrick & Reimer 1975; Silva-Benavides 1996; Gell *et al.* 2002; Soininen 2002), was identified from both Blind Roo A and Blind Roo B during each season, generally occurring in high numbers.

Despite some limited overlap between the dominant species of the circumneutral and alkaline wetlands (Groups 2 and 3), the overall community structure of the two groups of wetlands was found to be significantly different during both seasons analysed (winter and spring). Significant differences were also detected between the acidic Group 1 and circumneutral Group 2 wetlands during winter. However, the two groups had relatively similar species composition in spring. These similarities may have resulted from the fluctuation of sites between the acidic Group 1 and circumneutral Group 2, potentially contributing to changes in the overall community structure of the groups. Additionally, three of the four sites identified as Group 2 sites during spring were all close to the lower limit of the circumneutral classification, which may have influenced the type of diatoms present. A greater understanding of the community structure of circumneutral waters in this pH range could be gained from the inclusion of more circumneutral sites in future studies.

The findings generally suggest that pH is an important influence on diatom distribution, in accordance with other studies including ten Cate *et al.* (1993), Battarbee *et al.* (1997) and Siver *et al.* (2004). The results of the BIO-ENV analyses further support this concept with pH being identified as the variable most closely related to diatom community structure. It should however be noted that the relatively low number of study sites may have affected the reliability of the results. Larger sample sizes generally improve the chances of successfully linking biotic and environmental patterns (Clarke & Warwick 2001). Additionally, the influence of factors other than pH must be considered. For example the BIO-ENV results showed that the combination of pH and dissolved oxygen displayed a correlation similar to that of pH alone in each season. The effect of dissolved oxygen on species assemblages was highlighted by Schoeman (1973), who listed *Achnanthidium*, *Cymbella* and *Fragilaria* as taxa that are generally abundant in highly oxygenated waters.

A further consideration is the influence of variables which were not investigated during the study. Diatom community structure is known to be influenced by factors such as nitrogen (Saros *et al.* 2003), calcium (Patrick 1945) and metals (Anderson *et al.* 1986; Hirst *et al.* 2002; Gold *et al.* 2003), the latter of which is likely to be particularly relevant given the high levels of metals such as aluminium and iron commonly associated with acidic waters (Schindler 1988; Sammut & Lines-Kelly 2000). Accordingly, future studies would benefit from the inclusion of a greater number of environmental variables.

Seasonal differences in diatom community structure

Patrick (1964) reported that diatom assemblages may vary between seasons in terms of the taxa present and their contribution to overall community structure. Accordingly, there were some differences apparent in some of individual taxa during the current study. Despite this, the acidic sites were significantly different from the alkaline sites during each season and both pH groups contained some consistently dominant species. Taxa such as *Brachysira brebissonii* and *Frustulia magaliesmontana* were dominant in the Group 1 wetlands during each season while *Gomphonema parvulum* and *Staurosira construens* var. *venter* were consistently dominant in the Group 3 wetlands. Differences between the seasons were more evident for Group 2, potentially linked to the comparatively small pH range, resulting in greater variability in the number and proportion of sites classified as circumneutral in each season.

Conclusion

The overall structure of the diatom communities generally varied between the different wetland pH groups and pH was considered to be one of the factors responsible for variation in diatom community structure between regional localities. Furthermore, the results indicate that a relatively strong relationship between diatom community structure and pH was evident in various seasons, suggesting that diatoms would be useful biological monitors of acidification during a regular monitoring program. The study also identified potential indicator species for the various pH groups, however the application of these taxa as indicators requires further investigation.

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Appendix 1

Values for environmental variables measured at the 20 study sites from the south-west of Western Australia.

Site Name	Site Code	pH	Salinity	Electrical Conductivity	Temperature	Dissolved Oxygen	Vegetation Score
Bibra Lake	Su1	9.84	896.00	1588.00	20.40	4.64	4
	Wi1	8.48	324.00	764.00	14.90	7.50	4
	Sr1	8.23	663.00	1386.00	16.90	4.17	4
Black Diamond	Su2	5.63	132.00	325.00	31.00	5.35	3
	Wi2	5.73	193.00	538.00	12.80	7.21	3
	Sr2	5.26	195.00	447.00	19.00	8.27	3
Blind Roo A	Su3	6.45	60.30	124.80	29.30	6.76	3
	Wi3	6.64	73.80	202.50	14.80	5.42	3
	Sr3	5.86	115.00	249.00	17.70	5.72	3
Blind Roo B	Su4	10.00	242.00	473.00	32.60	7.56	2
	Wi4	9.08	187.00	495.00	15.10	5.49	2
	Sr4	9.29	187.00	395.00	21.90	10.18	2
Blue Gum Lake	Su5	9.65	944.00	1666.00	27.40	9.54	4
	Wi5	7.79	246.00	683.00	14.80	6.92	4
	Sr5	8.57	363.00	780.00	20.00	6.60	4
Blue Waters	Su6	4.03	594.00	1366.00	26.20	5.94	1
	Wi6	4.17	706.00	1866.00	12.90	7.52	1
	Sr6	3.98	686.00	1485.00	17.60	9.11	1
Ewington 2	Su7	4.25	555.00	1282.00	25.60	5.15	3
	Wi7	4.44	516.00	1381.00	12.90	7.32	3
	Sr7	4.16	637.00	1380.00	18.80	8.69	3
Exelby Wetland	Su8	8.22	151.00	302.00	32.00	6.19	2
	Wi8	7.01	154.00	410.00	15.30	4.01	2
	Sr8	7.19	201.00	423.00	22.30	7.15	2
Gnangara Lake	Su9	3.01	3140.00	5230.00	30.20	5.68	2
	Wi9	3.86	461.00	1172.00	15.50	7.30	2
	Sr9	3.59	802.00	1689.00	23.90	8.28	2
Herdsman Lake	Su10	8.88	397.00	729.00	25.40	13.50	3
	Wi10	7.66	361.00	923.00	15.60	5.02	3
	Sr10	8.04	523.00	1124.00	22.30	7.42	3
Knapping Wetland	Su11	9.46	337.00	637.00	34.60	7.26	3
	Wi11	7.28	267.00	697.00	15.60	5.14	3
	Sr11	9.30	289.00	603.00	24.20	11.03	3
Kurrajong Village Lake	Su12	6.54	165.00	316.00	26.10	9.03	2
	Wi12	6.30	111.00	315.00	16.40	5.40	2
	Sr12	6.73	177.00	392.00	20.20	8.81	2
Lake Monger	Su13	8.93	506.00	917.00	24.20	8.92	2
	Wi13	9.48	301.00	781.00	16.00	8.43	2
	Sr13	9.03	360.00	785.00	22.30	8.35	2
Lake Moyanup	Su14	8.24	142.00	284.00	30.50	4.95	3
	Wi14	7.50	104.00	281.00	14.80	6.04	3
	Sr14	8.81	168.00	355.00	22.90	10.70	3
Lakelands	Su15	6.15	1209.00	2086.00	24.80	5.67	4
	Wi15	6.73	481.00	1219.00	14.30	6.93	4
	Sr15	6.04	526.00	1125.00	22.50	8.27	4
Neil McDougall Park	Su16	9.78	270.00	503.00	20.90	11.87	2
	Wi16	7.66	44.10	130.80	15.90	6.04	2
	Sr16	8.50	78.00	179.50	19.90	7.57	2
Stockton Lake	Su17	5.53	185.00	447.00	27.10	5.21	3
	Wi17	6.45	187.00	523.00	13.10	7.47	3
	Sr17	4.59	258.00	582.00	17.70	9.02	3
Stockton Tailings Pond	Su18	3.31	451.00	1053.00	26.40	3.81	4
	Wi18	3.34	504.00	1350.00	13.00	7.64	4
	Sr18	3.12	618.00	1332.00	18.00	8.83	4
Tuscan Park	Su19	6.38	262.00	479.00	26.00	6.38	2
	Wi19	6.54	134.00	361.00	14.90	6.60	2
	Sr19	6.56	156.00	351.00	21.80	7.36	2
Wallsend Lake	Su20	6.49	177.00	430.00	25.90	5.93	4
	Wi20	6.92	179.00	503.00	12.70	7.32	4
	Sr20	6.66	195.00	447.00	19.00	8.27	4